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Presence of N-Acetylneuraminate Pyruvate-Lyase Activity in Sialidase Producing Strains of *Erysipelothrix rhusiopathiae* and *Oerskovia sp.*

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Abstract

The enzyme N-acetylneuraminate pyruvate-lyase (sialate aldolase) was found in two sialidase producing strains of the pathogen species *Erysipelothrix rhusiopathiae* and the saprophyte genus *Oerskovia.* The enzyme is secreted in the medium in both strains and its activity reaches similar values - 12.5 and 13.3 U/(mg protein) for *E. rhusiopathiae* B40 and *Oerskovia sp.*/14 respectively. Intracellular enzyme activity of 10.7 U/(mg protein) was also detected in the latter strain. The data obtained for the sialate aldolase and sialidase activities were compared to analogical data for *Vibrio cholerae* and *Aeromonas* sp. sialidase producer strains. The presence of sialate aldolase and sialidase enzymes in all these strains supports the concept that sialic acid metabolism contributes to the pathogenesis of microorganisms.
Key words: sialate aldolase, sialidase, *Erysipelothrix rhusiopathiae, Oerskovia sp.*

Резюме

Ензимът N-ацетил неураминат пируват-лиаза (сиалат алдолаза) е установен при два щама продуценти на сиалидаза – съответно от патогенния вид *Erysipelothrix rhusiopathiae* и сапрофитния род *Oerskovia.* И при двата щама ензимът се секретира извън клетката, като активността му има сходни нива – съответно 12.5 и 13.3 Е/(мг белтък). При щам *Oerskovia* sp./14 беше установена и вътреклетъчна сиалат алдолазна активност (10.7 Е/мг белтък). Данните, получени за продукцията на алдолаза и сиалидаза при двата изследвани щама са сравнени с аналогични данни за други сиалидаза продуциращи щамове (*Vibrio cholerae* и *Aeromonas sp.*). Наличието на сиалат алдолаза и сиалидаза при всички тези щамове подкрепя схващането за приноса на сиалометаболизма към патогенезата на микроорганизмите.

Introduction

N-acetylneuraminate lyases (4.1.3.3; N-acetylneuraminate pyruvate-lyases; sialate aldolases) are enzymes that reversibly split N-acetylneuraminic (sialic) acid into *N*-acetyl-D-mannosamine (ManNAc) and piruvate (Comb and Roseman, 1960). Sialic acids are a family of nine-carbon keto sugar acids, derivatives of N-acetylneuraminic acid (Neu5Ac). They are abundantly represented in the tissues of higher animals and irregularly in some of their microbial commensals or pathogens. Sialic acid metabolism is an object of interest for scientists in the areas of biochemistry, microbiology, medicine, etc, since sialoconjugates are involved in numerous cell-cell and cell-environment interactions. In bacteria, sialic acid metabolism is engaged in various functions, depending on microorganism's strategy for adaptation to certain ecological niche. The first step in the catabolism of these compounds is the cleavage of their terminal Neu5Ac residues (the reaction being catalyzed by sialidases), thus releasing free sialic acids. They are further degraded by sialic acid aldolases to form piruvate that enters either Embden-Meyerhof-Parnas pathway (as phosphoenolpyruvate) or citric acid cycle, and ManNAc which is converted to fructose-6-P for entrance into glycolysis (Vimr, 2004). Sialate aldolases also regulate the intracellular sialic acid concentration and prevent its rising to toxic levels (Vimr and Troy, 1985).

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According to BRENDA Enzyme Database (http://www.brenda-enzymes.org/), approximately 100 prokaryote species are reported to harbor sialidase genes. Since this enzyme is considered as a virulence factor, it has always been an object of thorough investigation. About 20 bacterial sialidases, most of them from pathogens *(Vibrio cholerae, Clostridium perfringens, Corynebacterium diphtheriae, Salmonella enterica, Bacteroides fragilis, Arthrobacter ureafaciens, Micromonospora viridifaciens, etc.),* are characterized in detail, the data including their full gene sequences, some crystal structures and 3D models. There are also commercial products of several proteins from well-known producer species.

Sialate aldolases are far less studied, though the number of species that have these enzymes identified as gene sequences is even slightly higher – more than 110. As for biochemical studies, there are some data of only about 15 bacterial species until now (Drzeniek *et al.*, 1972; Muller, 1973; Sanchez-Carron *et al.*, 2011, etc.).

Sialometabolism is related to the pathology of some bacterial species (Steenbergen *et al.*, 2005). Therefore, we find it intriguing to determine if sialate aldolases occur together with sialidase activity in *Erysipelothrix* and *Oerskovia* - genera, which aldolase activity has not been studied until now. The aim of this work is to investigate the presence, location and levels of aldolase and sialidase activity in two bacterial strains, representatives of these genera.

Materials and Methods

Strains, Media, and Cultivation Conditions

The sialidase-producing strains *Oerskovia sp.*/14 (O/14, NBIMCC №8734) (www.nbimcc.org) and *E. rhusiopathiae* B40 (B40) were tested for aldolase and sialidase activity. They were grown on Hottinger broth (Bulbio, NCIPD, Sofia, Bulgaria), pH 7.6, in 100/20 ml Erlenmeyer flasks on rotary shaker (50 r/min). All cultivations were for 24 h, at 37°C.

Enzyme assays

Sialidase and acylneuraminate pyruvate-lyase activities were measured quantitatively by colorimetric determination of free sialic acid by the thiobarbituric acid method, modified by Uchida *et al.* (1977). One unit of sialidase or aldolase activity was defined as the amount that releases or degrades 1 μg of N-acetylneuraminic acid (Neu5Ac) for 1 min at 37°C using glycomacropeptide (GMP) as a substrate. GMP is isolated by us from milk whey in laboratory conditions. The carbohydrate moiety of GMP includes galactose, N-acetylgalactosamine, and terminally linked sialic acids (predominantly Neu5Ac). The sialic acid content of GMP is 7–9 % (Abrashev *et al.*, 1980; Neelima *et al.*, 2013).

The extracellular and the cell-bound sialidase and sialate aldolase activities were assayed as either the supernatant or the biomass was used. The culture liquid was centrifuged at 3000 rpm for 20 min, and enzyme activities of the cell-free supernatant were determined. The cell pellet was suspended in phosphate buffer at pH 7.2 and homogenized. After centrifuging at 10,000 rpm for 15 min, the supernatant from disrupted cells was collected and subjected to further centrifugation at 14,500 rpm for 15 min. The supernatant (cell-free extract) was assayed for intracellular sialidase, sialate aldolase activities and protein content. The preparations of cell-free extracts were carried out at 0-4°C.

Protein concentrations were measured by the Lowry procedure (Lowry *et al.*, 1951), using bovine serum albumin as standard. Enzyme activities are expressed as units per mg protein [U/(mg protein)].

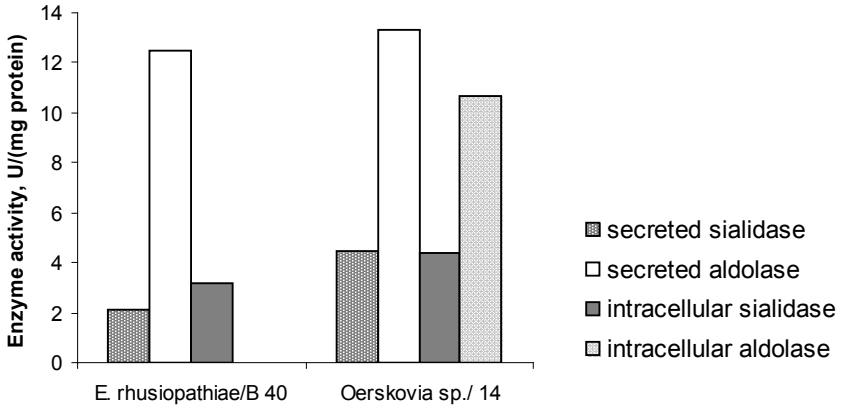

Fig.1. Levels of sialidase and aldolase activities in *E. rhusiopathiae* B40 and *Oerskovia sp.*/14

Results

The levels of secreted and cellular sialidase and aldolase activities of the strains are presented in Fig. 1. Both strains produced these enzymes, as their activities were higher in *Oerskovia*. Their extracellular aldolase activity was almost equal – 12.5 and 13.3 U/(mg protein) respectively for B40 and O/14. In *Oerskovia sp.*/14 we could also measure the intracellular activity of the enzyme – 10.7 U/(mg protein). Sialidase activity was registered in the culture liquids and the cell-free extracts in B40 and O/14. The levels of the secreted and the cellular sialidase activity in each strain were close. Interestingly, the presented activities were reached only when cultures were grown with agitation on rotary shaker, providing good aeration. When the same cultures were cultivated statically we could detect only traces of sialidase and aldolase activities (data not shown).

The data obtained in this study are compared to the analogical results, estimated earlier for two other strains – *Vibrio cholerae* non-O1/13 and *Aeromonas sp*. 40/02 (Table 1.). All the strains produce secreted sialidase. *V. cholerae* non-O1/13 does not produce extracellular aldolase. Intracellular aldolase in B40 could not be detected so far. The aeration conditions in which the production of sialidase and aldolase is expressed to the higher extend appear to be different for each strain.

Discussion

Sialidase has been reported for *E. rhusiopathiae* and *Oerskovia (Cellulomonas) turbata* (Abrashev and Orozova, 2006; Muller, 1995). Aldolase is reported for *E. rhusiopathiae* only as a sequence based annotation, while there are no data to our knowledge for occurrence of this enzyme in *Oerskovia*, until now.

As steps of a common metabolite pathway, sialidase and aldolase are met together in many cases (*C. perfringens, C. diphtheriae*, *V. cholerae, P. multocida, S. pneumoniae*), but there are also species that carry just one of them. For example, *E. coli* and *L. plantarum* do not produce sialidase. As can be concluded by our results, *E. rhusiopathiae* B40 and *Oerskovia sp.*/14 can be added to the list of species that carry both enzymes. It is noteworthy that most of these species, including *E. rhusiopathiae* B40, are pathogens. *Oerskovia* species are saprophytic nocardialike bacteria that have been associated with human infections only rarely, in cases of immunocompromised patients (Maguire *et al.*, 1996). It can be suggested that, in these cases the sialic acid metabolism acts in favor of pathogenesis, besides nutrition.

The aldolases of *Aeromonas sp*.40/02, *E. rhusiopathiae* B 40 and *Oerskovia sp.*/14 are secreted enzymes, indicating that these bacteria uptake ManNAc and piruvate after their production from sialic acid outside the cell and use them for energy metabolism (as is the case in *T. vaginalis* – Vimr, 2004). The relation of aeration conditions to the expression of sialidase was observed also in *V.cholerae* and *Aeromonas* 40/02. In the former species the mechanism of this phenomenon was explained recently by Liu *et al.* (2011). The authors reveal that the pathogenicity and virulence factors of *V. cholerae* are expressed at low oxygen concentrations. Since the four strains listed in the table show differences in this characteristic, it would be interesting to examine the possible relations between the expression of the enzymes of sialic acid metabolism and the conditions at which pathogenic mechanisms are revealed.

Table 1. Comparison of sialidase and aldolase production in the strains investigated in this study to analogical data from previous studies

Enzyme activity, (U/mg protein)		*E. rhusiopathiae* B40	*Oerskovia sp.*/ 14	*Aeromonas sp*. 40/02	*V. cholerae* non-O1/13
Neuraminidase	secreted	2.13	4.5	0.65	11.75
	cellular	3.2	4.4	4.9	4.2
Aldolase	secreted	12.4	13.3	1.9	-
	cellular	-	10.7	15.0	3.5
Aeration conditions		Aerobic on shaker	Aerobic on shaker	Aerobic static	Microaerophilic
Source		This study	This study	Engibarov *et al.*, 2015	Eneva *et al.*, 2011, 2015

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